PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2004/003511	12.08.2004	12.08.2003

International Patent Classification (IPC) or both national classification and IPC
C08B37/00, C07K17/12, A61K39/385, A61K47/48

Applicant
LIPOXEN TECHNOLOGIES LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/003511

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/GB2004/003511

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	3, 4, 8, 9, 20, 35
	No: Claims	1, 2, 5-7, 10-19, 21-34, 36-45
Inventive step (IS)	Yes: Claims	
	No: Claims	1-45
Industrial applicability (IA)	Yes: Claims	1-45
	No: Claims	

2. Citations and explanations

see separate sheet

Re Item IV

This Authority considers that there are two inventions covered by the claims indicated as follows:

I: *Claims 1-31* directed to a process for **producing an aldehyde derivative of a sialic acid compound** in which a starting material having a **sialic acid unit at the reducing terminal unit** is subjected to:

a)- reduction to form a vicinal diol group,

b)- selective oxidation to oxidise the vicinal diol group formed in step a) to form an aldehyde group.

and an **aldehyde derivative of a di-, oligo- or polysaccharide comprising at least one sialic acid unit**, a composition comprising such a compound and a diluent, and a pharmaceutical composition comprising a compound according to claims 25 or 28 and a pharmaceutically acceptable excipient.

The feature common to the claims 1-31 is the aldehyde derivative of claim 18.

II: *Claims 32-45* directed to a process in which a sialic acid starting material having a terminal **sialic acid unit at a non-reducing terminal end** is subjected to:

c)- selective oxidation to form an aldehyde group,

d)- reduction to reduce the aldehyde to the corresponding **alcohol**.

and a compound of formula II, being a derivative of mono-, di-, oligo- or polysaccharide, a pharmaceutical composition comprising a compound according to claim 43 and a pharmaceutically acceptable excipient, and a composition comprising a compound according to any of claims 38-43 and a diluent.

The feature common to the claims 32-45 is the compound of formula II of claim 38.

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows.

The prior art has been identified as document EP-A-0 454 898 and discloses a glycosaminoglycan-modified protein wherein an amino group of a protein is bound to an aldehyde group, which has been formed by reducing and partially oxidising the reducing terminal sugar moiety of a glycosaminoglycan such as colominic acid.

It follows that there is no common contribution over the prior art.

Also, examining the possible correspondence by technical effect, one finds that the technical effect of:

- the first invention is the **activation of the reducing end** of the sialic acid starting material to allow reaction with a protein,
- the second invention is the **desactivation of the non-reducing end** of the sialic acid starting material to avoid reaction with a protein.

This appears to show lack of corresponding technical effect as well. Consequently, neither the objective problem underlying the subjects of the claimed inventions, nor their solutions defined by the special technical features allow for a relationship to be established between the said inventions, which involves a single general inventive concept.

In conclusion, the groups of claims are not linked by common or corresponding special technical features and define two different inventions not linked by a single general inventive concept.

The application, hence, does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: EP-A-0 454 898 (SEIKAGAKU KOGYO CO LTD) 6 November 1991

D2: US-A-4 356 170 (JENNINGS HAROLD J ET AL) 26 October 1982

D3: US-A-5 097 020 (ANDERSON PORTER W ET AL) 17 March 1992

D4: GOUTAM SEN, CHITRA MANDAL: "The specificity of the binding site of Achatinin$_H$, a sialic acid-binding lectin from Achatina fulica" CARBOHYDRATE RESEARCH, vol. 268, 1995, pages 115-125, XP002303034

1. Novelty

1.1. The subject-matter of **claims 1, 2, 5-7, 10-19 and 21-31** is not novel over D1, D2

and D3 (Article 33(2) PCT).

D1 is directed to glycosaminoglycan-modified proteins wherein the amino group of the protein is bound to an aldehyde group formed by:

- reducing and thereby cleaving the reducing terminal sugar moiety of the glycosaminoglycan which can be colominic acid with an alkali boron hydride such as sodium boron hydride and sodium boron cyanohydride,
- followed by partially oxidising the reducing terminal sugar moiety using alkali periodates such as sodium periodate or potassium periodate (see page 5, lines 22-39, and claim 7).

The aldehyde compound is then reacted with an amino group of a protein by reductive amination (see page 5, lines 40-46). Pharmaceutical compositions containing said glycosaminoglycan-modified proteins together with a pharmaceutically acceptable carrier or diluent are also described (claim 9).

In D2, the reducing end group of an antigenic polysaccharide is made into the most susceptible site for oxidation by initially reducing it to its open chain hydroxyl form, the terminal non-reducing sialic residues containing vicinal hydroxyl groups being then oxidated to yield a reactive aldehyde group which is then covalently linked to a free amino group of a selected protein by reductive amination (see column 3, lines 8-39, column 4, lines 27-44, and claims 1, 2, 4, 6-8 and 16). The antigenic polysaccharide can be derived from Meningococci and E. coli, Meningococcal group B polysaccharide being disclosed in example 1.

D3 relates to the formation of reducing groups on the capsular polysaccharide like Neisseria meningitidis serogroup C (see column 2, line 7) by selective hydrolysis, e.g. by acids, bases or enzymes, combined with a specific oxidative cleavage, e.g. by periodate or related oxygen acids (see column 3, lines 63-65) to form aldehyde groups via which the capsular polysaccharide can be covalently attached to bacterial toxins or toxoids by means of reductive amination (see column 4, lines 22-62).

1.2. D4 anticipates the subject-matter of **claims 32-34 and 36-45** (Article 33(2) PCT).

D4 teaches that the oxidation of the trihydroxypropyl side chain of the sialic acid residue at the non-reducing end of the sialic acid-containing chain such as colominic acid, with periodate followed by borohydride treatment, i.e. reduction of the C-7 aldehyde group to a primary alcohol abolishes the inhibitory potency of said sialic acid compound towards

the sialic acid binding lectin ATN_H.

1.3. The subject-matter of **claims 3, 4, 8, 9, 20 and 35** is novel over the cited prior art (Article 33(2) PCT).

It seems that the crux of the present invention is to provide better defined products of protein conjugation with PSAs, the polysialic acid being monofunctional i.e. activated at the reducing end with an aldehyde group and passivated at the non-reducing end, thus avoiding unintended by-products during conjugation by giving rise to single-orientation attachment to proteins and avoiding the need to purify away to obtain pharmaceutically-acceptable conjugates.

It follows that the steps of:

- selective oxidation at the non-reducing end of the PSA,
- reduction at both the reducing end and the modified non-reducing end,
- selective oxidation at the modified reducing end,

are essential to the obtention of a compound which can be easily fractionated by ion exchange chromatography.

The Applicant should consider modifying its set of claims in order to better reflect the sought effect and thus also overcome the objection of lack of unity.

2. Inventive step

D1 is regarded as being the closest prior art to the subject-matter of claim 3.

The subject-matter of claim 3 differs from this known process in that an additional step of oxidising the vicinal diol group at the non-reducing end of the sialic acid-containing chain is performed prior to steps a) and b).

The technical problem to be solved by the present invention may therefore be regarded as to provide a process for the provision of a monofunctional polysialic acid which can be fractionated by ion exchange chromatography.

The skilled person, face with this technical problem, would have been prompted to combine the teaching of D1 and D4 to produce a monofunctional polysialic acid activated at the reducing end with an aldehyde group and passivated at the non-

reducing end without the exercise of inventive skill (Article 33(3) PCT).

The features of dependent **claims 4, 8, 9** are known. It would therefore be obvious to the person skilled in the art, to apply these features.

The compound of **claim 20** and the process of **claim 35** are obvious too.

3. Industrial applicability

The subject-matter of present **claims 1-45** appears to comply with the requirements of industrial applicability as stipulated in Article 33(4) PCT.